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# THE FERMENTATION OF XYLOSE BY BACTERIA OF THE AEROGENES, PARATYPHOID B. AND TYPHOID GROUPS \*

E. B. FRED AND W. H. PETERSON

*From the Departments of Agricultural Bacteriology and Agricultural Chemistry,  
University of Wisconsin, Madison*

In previous papers we have recorded results which show that xylose is fermented by *Lactobacillus pentoaceticus* with the production of almost equal amounts of acetic acid and lactic acid, and traces of alcohol and carbon dioxide. Approximately 80-90% of the sugar consumed may be accounted for by the two acids, acetic and lactic. Toward the aldohexose sugars, we noted these organisms behaved in a different manner; the end products are chiefly alcohol, lactic acid, carbon dioxide, and a small amount of acetic acid. Studies have since been made on the products of the fermentation of xylose by bacteria of the aerogenes-typhoid group, the results of which are presented in this report.

Of the various sugars used in fermentation tests to separate typhoid, paratyphoid A, and paratyphoid B, xylose has found especial favor. Weiss,<sup>1</sup> Stern,<sup>2</sup> Teague and Morishima,<sup>3</sup> and others have shown that xylose is fermented by *B. typhosus* without gas, by *B. paratyphosus* B with gas, and is not attacked by *B. paratyphosus* A. These groups of the typhoid organisms may be further subdivided into strains according to variations in the fermentation of xylose. In view of these facts and of the interest attached to the mode of decomposition of xylose, it was considered important to study the principal substances formed in the fermentation of this sugar. A comparison of these results with those obtained with *Lactobacillus pentoaceticus* presents marked differences in the end products.

The presence of volatile and nonvolatile acids, as well as alcohol, among the products of fermentation of glucose and other sugars by bacteria of the aerogenes-typhoid group has been noted by certain investigators. Harden<sup>4</sup> showed that the chief products of the fermentation of glucose by bacteria of the colon-typhoid group are lactic, succinic, acetic, and formic acids, ethyl alcohol, carbon dioxide, and hydrogen; the proportions of these substances varied with the different organisms. The typhoid bacteria produced a large

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<sup>1</sup> Jour. Med. Research, 1917, 31, p. 135.

<sup>2</sup> Centralbl. f. Bakteriol., I. O., 118, 82, p. 49.

<sup>3</sup> Jour. Infect. Dis., 1920, 27, p. 52.

<sup>4</sup> Jour. Chem. Soc., 1901, 79, p. 610.

amount of formic acid instead of a mixture of carbon dioxide and hydrogen. The colon bacteria produced only small amounts of formic acid but considerable quantities of carbon dioxide and hydrogen. The absence of carbon dioxide and hydrogen in the typhoid cultures is assumed to be due to their inability to ferment formates from which carbon dioxide and hydrogen are produced by the colon group. The lactic acid produced by *B. typhosus* was found to consist of about 47.5% of active acid. Unlike the fermentation with colon, he found that the growth of the typhoid organism was not vigorous and terminated when about half the sugar was fermented. The limited fermentation of glucose by *B. typhosus* and the production of both levulactic acid and inactive lactic acid was previously reported by Peré<sup>5</sup> in a paper in which he discussed the fermentation characteristics and products of *B. coli* and *B. typhosus*.

Harden and Walpole<sup>6</sup> found that *B. lactis-aerogenes* produces less acetic acid and more carbon dioxide than does *B. coli communis* in the fermentation of glucose. From the results of a detailed examination of the products, it was observed that only about two thirds of the carbon of the glucose was accounted for by the products lactic acid, acetic acid, succinic acid, formic acid, ethyl alcohol, carbon dioxide and hydrogen. In a search for other compounds, they found a considerable quantity, approximately 33% of the carbon of the sugar appeared as 2:3 butyleneglycol and a very small amount of acetylmethylcarbinol. In a later report by Harden and Norris,<sup>7</sup> it was found that *B. lactis aerogenes* produces acetylmethylcarbinol and 2:3 butyleneglycol in the fermentation of glucose, fructose, mannose, galactose, arabinose, isodulcitol, mannitol, or adonitol.

Duchacek<sup>8</sup> studied the products of the fermentation of glucose by *B. coli* and *B. typhosus* in cultures exposed to the atmosphere and to an atmosphere of hydrogen. In agreement with Harden and his associates, Duchacek found that the colon organism ferments glucose much faster and much more completely than the typhoid. In cultures 11 days old exposed to the air, about 53% of the glucose was fermented by *B. coli* and about 18% by *B. typhosus*. In the cultures exposed to the air the fermentation proceeded faster than in an atmosphere of hydrogen. The chief products of fermentation by these organisms were two organic acids, lactic and acetic. With *B. coli*, carbon dioxide was produced in large amounts in addition to the acids. According to Sera<sup>9</sup> the fermentation of glucose by *B. typhosus* results in the production of acetic acid, formic acid, and a trace of alcohol. No attempt was made to measure other products of fermentation.

#### EXPERIMENTAL

The fermentations were in every case carried out in a 300 cc Erlenmeyer flask connected to carbon dioxide traps; 250 cc of culture medium was used. The exact arrangement of the apparatus and the methods of examining the products of fermentation have previously been described in detail.<sup>10</sup> The medium consisted of a fresh yeast water extract, containing 0.5% dibasic potassium phos-

<sup>5</sup> Ann. Inst. Pasteur, 1892, 6, p. 512.

<sup>6</sup> Proc. Roy. Soc., Series B, 1906, 77, p. 399.

<sup>7</sup> Proc. Roy. Soc., Series B, 1912, 84, p. 492.

<sup>8</sup> Centralbl. f. Bakteriologie, I, O., 1904, 37, p. 161 and p. 526.

<sup>9</sup> Ztschr. f. Hyg. u. Infektionskr., 1910, 66, p. 162.

<sup>10</sup> Fred, E. B.; Peterson, W. H., and Davenport, Audrey, Jour. Biol. Chem., 1919, 39, p. 347; 1920, 41, p. 431.

phate and 0.5% of Difco peptone. To this was added 2% of xylose and the medium was then sterilized in the autoclave at 15 lbs. pressure for 30 minutes. At the time of inoculation, a few drops of bromocresol purple were added and the cultures incubated at 37 C. The acids formed during the fermentation, as shown by the indicator bromocresol purple, were neutralized with sterilized 1 N NaOH. In all experiments uninoculated controls were made and the results of their analyses were subtracted from the results of the inoculated flasks. Although the inoculated cultures without sugar showed a certain amount of fermentation, it was decided not to subtract these results from those of the sugar cultures. Harden<sup>4</sup> and others have shown that the fermentation of peptone in the absence of sugar is different from that in the presence of sugar.

The chief products formed by the action of *B. aerogenes*, culture 26, *B. typhosus*, and *B. paratyphosus* B on xylose are given in this report. The strains of *B. typhosus* and *B. paratyphoid* B used were furnished by the Army Medical School. The *B. lactis aerogenes* and culture 26 were taken from our laboratory stock cultures. These two organisms were isolated from silage and are no doubt closely related strains. The chief biologic characteristics of *B. lactis aerogenes* and of culture 26 are clearly seen in the following table in which + means acid and gas; and — no fermentation.

TABLE 1  
BIOLOGIC CHARACTERISTICS OF *B. LACTIS AEROGES* AND CULTURE 26

	<i>B. lactis aerogenes</i>	Culture 26		<i>B. lactis aerogenes</i>	Culture 26
1. Arabinose.....	+	+	11. Raffinose.....	+	+
2. Xylose.....	+	+	12. Melezitose.....	—	—
3. Rhamnose.....	+	+	13. Mannitol.....	+	+
4. Glucose.....	+	+	14. Glycerol.....	—	—
5. Fructose.....	+	+	15. Salicin.....	+	+
6. Galactose.....	+	+	16. Esculin.....	+	+
7. Mannose.....	+	+	17. Inulin.....	—	—
8. Sucrose.....	+	+	18. Starch.....	—	—
9. Lactose.....	+	+	19. Sodium lactate..	—	—
10. Maltose.....	+	+			

These two organisms show a close resemblance to each other; they are rod forms, motile, gram-negative, nonliquefying, and give a positive Voges-Proskauer reaction. They ferment carbohydrates vigorously with the production of much alcohol, carbon dioxide, and hydrogen. The reaction of the medium becomes acid and later reverts to a lower degree of acidity.

The cultural reactions of the four organisms—culture 26, *B. lactis aerogenes*, *B. typhosus*, and *B. paratyphosus* B—were studied in fermentation tubes containing the xylose-peptone-yeast water. Since gas measurements in these tubes, especially carbon dioxide, are not satisfactory, the usual method of procedure was modified. Twenty-four hours after the tubes were inoculated, about 2 c c of sterilized mercury was added; just enough mercury to seal the tube at the lowest point, is sufficient. This modification of the Smith fermentation tube was tested with many different organisms. It was found that the addition of mercury checked the diffusion and escape of carbon dioxide from the fermentation tube. The presence of mercury in these tubes exerts a slightly retarding effect on the growth of the bacteria and it is, therefore, advisable to allow the inoculated cultures to grow for at least 24 hours before adding the mercury. The effect of mercury on the retention of carbon dioxide may be seen from the following results:

	CO <sub>2</sub> from 12 c c of culture
No mercury.....	0.0222 gm.
Mercury, 2 c c.....	0.1000 gm.

In this test, the tubes of 2% glucose yeast water were inoculated with a pure culture of pentose fermenters and 24 hours later mercury was added. After 2 weeks the carbon dioxide in the long arm of the tube was fixed with potassium hydroxide and determined by the Van Slyke apparatus. The value of the mercury seal in the retention of carbon dioxide is clearly seen from the results of these analyses. In a similar experiment, the gas retained in the tubes at varying intervals of time was measured.

TABLE 2  
GAS RETAINED IN TUBES AT VARYING INTERVALS OF TIME

	Gas Retained After			
	2 Days, C c	6 Days, C c	12 Days, C c	18 Days, C c
Culture 26, no mercury.....	6.0	7.0	6.0	5.0
Culture 26, mercury.....	7.0	9.0	9.0	9.0
<i>B. lactis aerogenes</i> , no mercury.....	4.5	4.5	3.5	2.0
<i>B. lactis aerogenes</i> , mercury.....	3.5	4.5	10.5	11.0

Here it will be seen that the gas is gradually lost by diffusion through the liquid. In the presence of mercury there is no indication of loss, but often a continued gain in the quantity of gas.

Absorption tests of the gas collected in the closed arm of the fermentation tube showed that from xylose, culture 26 produces about 2 parts of carbon dioxide to one part of hydrogen, while *B. typhosus* does not produce any gas. On the other hand, *B. paratyphoid B* decomposes xylose in a manner similar to that of the aerogenes organisms. The ratio of gas is about 2 parts of carbon dioxide to 1 part of hydrogen.

THE FERMENTATION OF XYLOSE BY *B. LACTIS AEROGENES*, CULTURE 26, *B. TYPHOSUS*, AND *B. PARATYPHOSUS B*.

The substances produced by the action of *B. lactis aerogenes* and culture 26 on xylose were found to be small amounts of volatile and nonvolatile acids, and large amounts of ethyl alcohol, carbon dioxide and hydrogen. The sum of the products, with the exception of hydrogen which was not determined quantitatively, reveals the fact that only about two thirds to three fourths of the xylose is accounted for by these end products. The results of the analyses are shown in the figures of table 3. The yield of volatile and non-volatile acids was so small that the kind of acid could not be determined. By far the larger part of the decomposed sugar is accounted for by the end product, carbon dioxide. The percentages by weight of the products from the fermentation of xylose by these two organisms are given in table 4.

TABLE 3  
THE PRODUCTS OF THE FERMENTATION OF XYLOSE BY BACTERIA OF THE AEROGENES  
TYPHOID GROUP CALCULATED FOR 100 C C OF CULTURE

Products	Culture 26 Gm.	<i>B. lactis</i> <i>aerogenes</i> , Gm.	<i>B. para-</i> <i>typhoid</i> B7, Gm.	<i>B. para-</i> <i>typhoid</i> B8, Gm.	<i>B. ty-</i> <i>phoid</i> 11, Gm.	<i>B. ty-</i> <i>phoid</i> 15, Gm.
Ethyl alcohol.....	0.4195	0.4458	0.3878	0.3070	0.1217	0.1198
Formic acid.....	.....	.....	0.0307	0.0862	0.1023	0.1045
Acetic acid.....	0.0513	0.0777	0.3101	0.3651	0.1590	0.1517
Butyric acid.....	.....	.....	0.0862	0.0741	0.0290	0.0166
Lactic acid.....	0.0156	0.0000	0.1045	0.1678	0.0000	0.0000
Succinic acid.....	.....	.....	0.6861	0.4521	0.1312	0.1171
Carbon dioxid.....	0.7173	0.9446	0.3051	0.4010	0.0408	0.0308
Total weight of products....	1.2037	1.4681	1.9105	1.8533	0.5840	0.5405
Sugar unfermented.....	0.1611	0.1056	trace	trace	1.5984	—

The products obtained from the fermentation of xylose by *B. paratyphoid B* are formic, acetic, butyric, lactic, and succinic acids, ethyl alcohol, carbon dioxide, and hydrogen. Here the sum of the products formed are greater than those obtained from the fermenta-

tation of xylose by the two strains of aerogenes. In a 2% solution of xylose these paratyphoid B organisms fermented the sugar completely and the sum of the products represents more than 92% of the original sugar. The ethyl alcohol and carbon dioxide are produced in nearly equal quantities. The two strains of paratyphoid B show a variation in the amounts of volatile acid formed; culture 8 produces somewhat larger amounts of volatile acid. Unlike the aerogenes group, these organisms form large quantities of nonvolatile acid which consist of succinic acid and lactic acid. The percentage relations of these acids are given in table 4.

TABLE 4  
THE PRODUCTS OF THE FERMENTATION OF XYLOSE BY BACTERIA OF THE AEROGENES  
TYPHOID GROUP CALCULATED FOR 100 C C OF CULTURE

Products	Culture 26, Percentage	B. lactis aerogenes, Percentage	B. paratyphoid B7, Percentage	B. paratyphoid B8, Percentage	B. typhoid 11, Percentage	B. typhoid 15, Percentage
Ethyl alcohol.....	34.86	30.36	20.30	16.57	20.84	22.16
Formic acid.....	.....	.....	1.61	4.65	17.51	19.33
Acetic acid.....	4.26	5.29	16.23	19.70	27.23	28.07
Butyric acid.....	.....	.....	4.51	4.00	4.96	3.07
Lactic acid.....	1.29	0.00	5.47	9.05	0.00	0.00
Succinic acid.....	.....	.....	35.91	24.39	22.47	21.67
Carbon dioxid.....	59.59	64.35	15.97	21.64	6.99	5.70
<b>Total.....</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>

In the fermentation of xylose by the typhoid bacteria the products are formic, acetic, butyric, and succinic acids, ethyl alcohol, and very small amounts of carbon dioxide. The sum of the products formed, as well as the results of sugar analyses, show that these organisms utilize only a small part of the sugar, approximately 25% of the xylose in the medium. Somewhat similar results have been secured by Harden <sup>4</sup> for glucose; no gaseous products except carbon dioxide are formed, and this only in small amounts. In this respect the action of *B. typhosus* on xylose resembles that of *Lactobacillus pentoaceticus*. Just why the typhoid bacteria make use of such a small portion of the sugar is not known.

From a comparison of the products of fermentation obtained from xylose by the action of these different bacteria it will be clearly seen that they are widely separated as regards their biochemical properties. Although commonly discussed under the class name, colon-typhoid group, they exhibit marked differences both quantitatively and qualitatively in their action on xylose. The aerogenes forms attack this pentose sugar with the production of by-products

similar to those obtained in the alcoholic fermentation by yeasts. The paratyphoid B organisms are distinguished by their vigorous fermentation with the production of acids, particularly large amounts of succinic acid. In contrast to these two groups, the typhoid bacteria attacks xylose slowly and form only small amounts of acid, ethyl alcohol, and traces of carbon dioxide.

#### IDENTIFICATION OF PRODUCTS

*Volatile Acid and Alcohol.*—After the volatile acid from the steam distillation was titrated with 0.1 N barium hydroxide, the distillate was evaporated to dryness on the steam bath, taken up with 60-70 c c of hot water and filtered into 300 c c Erlenmeyer flasks. The acid was set free from the barium salt by the addition of the theoretical amount of 1 N sulphuric acid. The acid was added through a filter funnel drawn out to a capillary tube. After standing over night the barium sulphate was filtered and washed, and the filtrate and washings were made to 110 c c with carbon dioxide free water. This solution was then subjected to a Duclaux distillation and the distilling constants calculated from the titration data. In the case of alcohol, the acid formed by oxidation and subsequent distillation was subjected to the same manipulation. The distilling constants obtained are given in table 5. For comparison Duclaux's distilling constant for acetic acid is also given. Qualitative tests on the combined distillate and residue from the Duclaux procedure showed the presence of formic, acetic, and butyric acids. These acids were therefore present in such amounts as to give a distilling constant closely approaching that of acetic acid. For the two typhoid cultures the constants are slightly lower than for acetic acid, which indicates the presence of formic acid. The constants for alcohol run somewhat high in all cases, but as no evidence for the presence of a lower alcohol than ethyl was found, the quantity of higher alcohol present must be small.

The barium salts from the Duclaux distillation were evaporated to dryness, taken up with water and made to a volume of 100 c c. An aliquot was used for the determination of formic acid by the Fincke<sup>11</sup> method. The data obtained are given in table 6 and show that a large part of the volatile acid is formic acid. A strong reduction of silver nitrate also indicated the presence of formates.

<sup>11</sup> Biochem. Ztschr., 1913, 51, p. 253.



Another aliquot was evaporated to dryness in a platinum dish, dried at 130 C. for several hours, and the weight of the barium salts determined. These salts were ignited in the presence of an excess of sulphuric acid and the weight of barium sulphate equivalent to the organic salts was obtained. The data are given in table 7 and show that the volatile acid is a mixture of acids. The data are in general agreement with the Duclaux distilling constants and indicate the formation of some acid higher than acetic by the two paratyphoid organisms, and the formation of a lower acid by the typhoid bacteria. In view of the large amounts of formic acid found some higher acid, such as butyric, is required to give the percentages of barium sulphate and the Duclaux distilling constants obtained from the products of fermentation. A strong odor of butyric acid was easily detected when sulphuric acid was added to the barium salts before igniting them.

TABLE 5  
DISTILLING CONSTANTS OF THE VOLATILE ACIDS AND ALCOHOLS OBTAINED BY THE  
DUCLAUX METHOD

Culture		10 C c	20 C c	30 C c	40 C c	50 C c	60 C c	70 C c	80 C c	90 C c	100 C c
Duclaux distilling constants for acetic acid		7.4	15.2	23.4	32.0	40.9	50.5	60.6	71.9	84.4	100
Culture 26.....	Alcohol.....	7.8	15.9	24.3	33.1	42.3	51.8	62.1	73.1	85.5	100
B. lactis aerogenes.....	Alcohol.....	7.7	15.8	24.3	33.0	42.2	51.8	62.1	73.1	85.5	100
B. paratyphoid B7.....	Volatile acid..	7.6	15.5	23.9	32.7	41.7	51.3	61.4	72.6	85.0	100
B. paratyphoid B7.....	Alcohol.....	7.8	15.9	24.4	33.2	42.3	51.9	62.1	73.2	85.5	100
B. paratyphoid B8.....	Volatile acid..	7.4	15.2	23.5	31.8	40.9	50.4	60.7	71.9	84.7	100
B. paratyphoid B8.....	Alcohol.....	7.8	16.0	24.4	33.2	42.4	52.1	62.3	73.4	85.7	100
B. typhoid 11.....	Volatile acid..	7.3	14.8	23.0	30.9	39.7	49.0	59.2	70.5	83.6	100
B. typhoid 11.....	Alcohol.....	7.7	16.1	24.6	33.5	42.7	52.3	62.5	73.5	85.6	100
B. typhoid 15.....	Volatile acid..	7.2	14.5	22.7	30.9	39.6	49.0	59.1	70.4	83.5	100

TABLE 6  
THE FORMIC ACID CONTENT OF THE BARIUM SALTS OF THE VOLATILE ACIDS

Culture	Barium Salts of Volatile Acids as 0.1 N, C c	Weight of HgCl, Gm.	Formic Acid Equivalent to HgCl, Gm.	Formic Acid Calculated for 100 c c of Cul- ture, Gm.
B. paratyphoid B7.....	43.5	0.1962	0.0191	0.0307
B. paratyphoid B8.....	39.5	0.3670	0.0358	0.0862
B. typhoid 11.....	24.7	0.4968	0.0487	0.1023
B. typhoid 15.....	37.6	0.7682	0.0749	0.1045

After due allowance is made for the formic acid, an approximate estimate of the amounts of acetic and butyric acids can be calculated from the percentage of barium sulphate. The data given in table 3 have been calculated in this way from the analytical data of tables 6 and 7.

*Nonvolatile Acid.*—The acids extracted by ether from the residue of the steam distillation were titrated with 0.1 N barium hydroxide after the addition of 30-40 c c of water and removal of the ether by distillation. With *B. paratyphoid* B 8 it was noted that crystals had separated out from the ether before the extraction flask was disconnected from the extractor. These crystals dissolved readily in water added to the ether before the titration was made.

TABLE 7  
COMPOSITION OF THE BARIUM SALTS OF THE VOLATILE ACID

Culture	Barium Salts of the Volatile Acid, Gm.	Barium Sulphate Found	
		Gm.	Percentage
<i>B. paratyphoid</i> B7.....	0.5522	0.4942	89.50
<i>B. paratyphoid</i> B8.....	0.3718	0.3406	91.61
<i>B. typhoid</i> 11.....	0.3056	0.2886	94.44
<i>B. typhoid</i> 15.....	0.4314	0.4116	95.41
Theory for barium formate.....			102.64
Theory for barium acetate.....			91.37
Theory for barium butyrate.....			74.91

The barium salts of the nonvolatile acid were evaporated to dryness on the steam bath and then fractionated into the salts of lactic and succinic acids. For this purpose the dried salts were extracted with 10-20 c c of water, filtered into graduates, and absolute alcohol added until the concentration of alcohol was 90% by volume. A flocculent precipitate was formed and after standing in the refrigerator for a day or two, it was filtered off and washed with 90% alcohol. By this procedure the barium succinate is precipitated while the barium lactate remains dissolved in the alcohol. The greater part of the dried salts remained undissolved in the presence of a small amount of water and were later used for the determination of their barium content.

The alcohol filtrate containing the barium lactate was evaporated to a small volume, diluted with 75-100 c c water and 0.2 N zinc sulphate added in small portions until no more barium sulphate was precipitated. After standing on the steam bath over night, the barium sulphate was filtered off, ignited, and weighed; from its weight the equivalent quantity of lactic acid was calculated. The barium salt remaining undissolved after the barium lactate was extracted, was transferred to a platinum dish, dried for 4 hours at 125 C. and weighed. It was then transferred to a beaker with about 100-150 c c of water. The water was heated to boiling and an excess of sulphuric

acid added to decompose the salt and to precipitate the barium as barium sulphate. In order to insure complete decomposition, the mixture of barium sulphate, undecomposed salt, and sulphuric acid was thoroughly triturated with a blunt glass rod for 10-15 minutes at the boiling point of the liquid. The beaker was left on the steam bath over night and in the morning the barium sulphate was filtered off, washed, ignited, and weighed. From the weight of barium sulphate obtained, the barium content of the unknown salt was found and proved to agree closely with that required for barium succinate. The data are given in table 8.

TABLE 8  
BARIUM CONTENT OF SUCCINIC ACID PRODUCED IN THE FERMENTATION OF XYLOSE

Origin	Weight of Barium Salt, Gm.	Weight of Barium Sulphate Found, Gm.	Percentage of Barium	Percentage of Barium in $(\text{CH}_2)_2(\text{CO}_2)_2\text{Ba}$ ,
B. paratyphoid B7.....	1.5918	1.4472	53.6	53.8
B. paratyphoid B8.....	0.5464	0.4936	53.3	53.8
B. typhoid 11.....	0.1780	0.1600	53.1	53.8

The excess of sulphuric acid in the solution containing the free succinic acid was precipitated with barium hydroxide, care being taken not to add an excess of barium hydroxide. After the barium sulphate was filtered off, the solution of succinic acid was evaporated to a small volume on the steam bath and the succinic acid crystallized out by concentrating in a desiccator over sulphuric acid. A few crystals were treated with ammonia and zinc dust according to Neuberg's<sup>12</sup> test for succinic acid. The vapors of the fused mass produced a deep red color on a pine splinter moistened with hydrochloric acid which is characteristic for succinic acid. This qualitative test, coupled with the barium content and crystalline appearance of the free acid, clearly established the identity of the acid as succinic.

From the foregoing data, the quantity of lactic and succinic acids contained in the nonvolatile acid has been calculated and is given in table 3. In these calculations the lactic acid is found by direct determination and the succinic acid obtained by subtracting the lactic acid from the total nonvolatile acid. That this procedure leads to correct results is indicated by the data of table 8. After the barium lactate,

<sup>12</sup> Ztschr. f. physiol. Chem., 1901, 31, p. 574.

together with a small amount of barium succinate, was extracted, the insoluble residue of nonvolatile acid was found to be barium succinate.

#### SUMMARY

Xylose in yeast water peptone solutions is readily fermented by bacteria of the aerogenes and paratyphoid B groups. These organisms break up the xylose with a rapid evolution of gas. The products of fermentation with *B. lactis aerogenes* are essentially carbon dioxide, hydrogen, and alcohol; in this respect the aerogenes forms are somewhat similar to the yeasts. In addition to these products, small amounts of volatile acid are found. The two substances, carbon dioxide and ethyl alcohol, represent about 75% of the sugar consumed. In relation to reaction, the aerogenes organisms produce acid, at first, until the medium is about  $P_H$  4.4; later this reaction reverts to an approximate  $P_H$  5.0. The destruction of the sugar takes place rapidly.

The main products formed in the fermentation of xylose by paratyphoid B are formic, acetic, butyric, lactic, and succinic acids, ethyl alcohol, carbon dioxide, and hydrogen; these products represent about 92% of the original sugar. Xylose is fermented rapidly and almost completely by the paratyphoid B. organisms. In agreement with the aerogenes bacteria, these organisms form large amounts of alcohol and carbon dioxide.

The fermentation of xylose by the typhoid bacteria is far from complete. In general not more than one fourth of the xylose is decomposed. No gaseous products except small amounts of carbon dioxide were found. The chief substances are alcohol; formic, acetic, butyric, and succinic acids; and a trace of carbon dioxide. The greater part of the fermented xylose is represented by the succinic acid.

It is clearly shown from the results of this work that xylose is attacked by the organisms of the aerogenes-typhoid group with the production of volatile, nonvolatile, and gaseous substances. Although the organisms included in this study are placed in the same group, their by-products differ quantitatively and qualitatively.